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Physiological and Respiratory Responses of the Mozambique Tilapia (*Oreochromis mossambicus*) to Salinity Acclimation

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ABSTRACT. We have examined several physiological variables related to salinity acclimation in the euryhaline tilapia, *Oreochromis mossambicus*. Tilapia reared in fresh water (FW) were transferred to FW, isosmotic salinity (ISO, 12‰) and 75% seawater (SW, 25‰). Oxygen consumption, plasma levels of cortisol, growth hormone (GH), prolactins ($tPRL_{177}$ and $tPRL_{188}$), glucose, ions (Na^+, K^+, Cl^-), and gill Na^+,K^+-ATPase activities were measured for up to 4 days in each salinity treatment. Plasma Na^+ and Cl^- concentrations were elevated 1 day after transfer to SW, but returned to FW values on day 4. Plasma cortisol and glucose levels were higher in FW and ISO than in SW 1 day after transfer. Plasma GH levels of tilapia in SW increased above FW and ISO values after 4 days, whereas plasma PRL levels decreased in ISO and SW compared to FW at 4 days. These results are consistent with the possible osmoregulation roles of GH and PRL in SW and FW, respectively. Gill Na^+,K^+-ATPase activity of tilapia in SW increased more than 2-fold over the FW value after 4 days, but activity of this enzyme did not change in ISO. Oxygen consumption rates of tilapia in SW were significantly elevated 4 days after transfer compared to FW and ISO. The results of this study indicate that the physiological changes associated with SW acclimation in tilapia represents a significant short-term energetic cost, and may account for as much as 20% of total body metabolism after 4 days in SW. COMP BIOCHEM PHYSIOL 117A;3:391–398, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Cortisol, gill Na^+,K^+-ATPase, growth hormone, osmoregulation, oxygen consumption, prolactin, salinity, tilapia

INTRODUCTION

The Mozambique tilapia (*Oreochromis mossambicus*) is a euryhaline cichlid which has been introduced from its native Africa to tropical freshwater (FW) and marine environments around the world (7). This species has provided a good model for studying the mechanisms of osmoregulation in teleost fishes (e.g., 21,22), due to its euryhaline nature and hardiness in captivity (18).

There have been many studies on the physiological changes that occur during the seawater (SW) acclimation process in *O. mossambicus*. Following transfer from FW to SW there is a temporary elevation in plasma osmolarity and sodium ($[Na^+]$) and chloride ($[Cl^-]$) ion concentrations (2,26) accompanied by a transient rise in plasma cortisol concentrations (4), and a more gradual increase in plasma growth hormone (GH) levels (49). There is an alteration in branchial chloride cell morphology (25) and an increase in gill Na^+,K^+-ATPase activity (16,17,26) in SW. Furthermore, there is recent evidence to link the actions of cortisol and GH to these processes in *O. mossambicus* (11,31). Tilapia possess two forms of prolactin ($tPRL_{177}$ and $tPRL_{188}$) (44,50), which both have a sodium retention effect in FW and thus decrease to low levels in the plasma of fish transferred to SW (6,49). The result of these biochemical and hormonal responses is a net efflux of Na^+ and Cl^- to maintain ionic balance in a hyperosmotic environment (17,36).

The metabolic response of tilapia during the SW acclimation process is less clear. Oxygen consumption has commonly been used as an indirect indicator of metabolism in fish (14), and measurements of oxygen consumption rates in different salinities have been employed in an attempt to determine the energetic cost of osmoregulation in tilapia. Farmer and Beamish (19), using the Nile tilapia (*Oreochromis niloticus*), and Febry and Lutz (20), using the Florida red hybrid tilapia (*O. mossambicus* × *Oreochromis hornorum*), found that oxygen consumption rates of swimming fish were lowest in isosmotic salinity (ISO; 12‰) com-

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pared to FW and SW. In contrast, Job (28) reported ISO to have the highest rate of oxygen consumption in *O. mossambicus*, but activity was not controlled in that study and therefore statements regarding osmoregulatory costs cannot be made unequivocally. Furthermore, the fish in the three studies above were acclimated to the test salinities for at least 1 month before oxygen consumption rates were determined, and therefore do not reflect metabolic requirements of the animals during the acclimation process.

The purpose of the present study was to examine the physiological and respiratory responses of *O. mossambicus* during the acclimation to ISO and SW. Simultaneous measurements of oxygen consumption rates, plasma constituents and gill Na^+,K^+-ATPase activities were carried out in tilapia after transfer from FW to FW, ISO, and SW.

MATERIALS AND METHODS

Fish

Adult male and female *O. mossambicus* maintained in FW at the Hawaii Institute of Marine Biology (Coconut Island, Hawaii) were used in the study. These fish originated from a population on Oahu that was introduced to Hawaii from Singapore in 1951 (23,47). While in captivity, they were kept in a 5000-l circular fibreglass tank under a natural photoperiod (approx. 14 hr light : 10 hr dark). The fish were fed a daily diet of Purina trout chow during the experiment, but were not fed for 24 hr before sampling.

Salinity Exposure

In April 1994, tilapia (50–80 gm) were transferred randomly into three 60-l oval fiberglass tanks at a density of 25 fish per tank. The fish were checked to ensure that no brooding females were used in the experiment. One tank was supplied with FW and two contained ISO [12‰; (20)]. The salinity in one of the ISO tanks was then increased over the next 6 hr to 75% SW (25‰). An initial attempt to acclimate fish to 100% SW (34‰) over 30 hr resulted in 92% mortality. Previous studies have shown that *O. mossambicus* cannot tolerate an abrupt transfer from FW to full-strength SW, and require a gradual (1 week) acclimation period [see review in (45)]. Water temperatures were kept similar in each treatment tank (22 ± 1°C) and aeration was provided to maintain dissolved oxygen levels above 95% saturation. Water samples were collected to measure the ionic composition of each salinity treatment (Table 1). Eight fish were sampled prior to the transfer, and at 1 and 4 days after transfer to the treatment tanks. The fish were anesthetized in 2-phenoxyethanol (1 ml l^{-1}), killed by a blow to the head, and blood was collected from the caudal vessels using heparinized syringes (24). The blood samples were centrifuged (2000 **g** for 5 min) and the plasma was removed and stored at −75°C for later analyses of cortisol, GH, the two PRLs, glucose, and ions. Immediately following blood collection, gill filaments were removed from the second gill arch on the left side of the fish, placed in 1 ml ice-cold sucrose buffer (0.3 M sucrose, 0.02 M Na_2EDTA, 0.1 M imidazole, pH 7.1), and stored at −75°C for measurement of Na^+,K^+-ATPase activity.

TABLE 1. Chemical composition of water samples collected from the salinity treatment tanks (FW = fresh water, ISO = isosmotic water, SW = seawater)

	Medium			
Variable	**FW**	**ISO**	**75% SW**	**SW**
Salinity (‰)	0	12	25	34
Na^+ (mM)	2	161	322	482
Cl^- (mM)	<1	182	377	553
K^+ (mM)	BDL[a]	3.0	6.9	9.2
Ca^{2+} (mM)	0.4	3.4	6.7	8.4
Mg^{2+} (mM)	0.7	15.8	27.9	35.2

[a]Below detection limits.

Respirometry

Oxygen consumption rates of tilapia in the salinity treatments were measured at the last sampling period (4 days after transfer) using a respirometer which consisted primarily of a plexiglass swimtube (14). The total volume of the respirometer was 2.75 l and the swimtube was 36 cm long and 8.5 cm in diameter. Water flow was generated using a centrifugal pump (Eheim model 1250) connected to the swimtube with vinyl tubing. A valve assembly allowed the respirometer to operate in a flow-through (acclimation) or closed (measurement) mode. Water oxygen concentrations were measured using a dissolved oxygen meter (Oxyguard® Mk III, Point Four Systems, Port Moody, British Columbia) with the electrode mounted inside the respirometer. The oxygen electrode was calibrated in air to 101% saturation before use, according to meter's operating manual.

Prior to each respirometry trial, individual fish from a treatment tank were introduced to the swimtube and allowed to acclimate in flow-through water. The fish were not fed for 24 hr prior to testing, in order to ensure a postabsorptive digestive state (10). The respirometer was covered with black plastic throughout acclimation and testing to shield the fish from visual disturbances. In each trial, the swimming speed was set to 0.5 body length per sec to standardize the level of activity (9). After acclimation, the respirometer was closed and the subsequent decline in water oxygen concentration (to the nearest 0.1 mg l^{-1}) was monitored at 5-min intervals for 20 min. After a trial was completed, the fish was removed from the swimtube and its length and weight measured. To adjust for possible bacterial oxygen consumption within the system, blank trials without any fish were run throughout the experiment. Water temperatures during the trials were kept similar to the treatment tanks (mean 23°C).

Water oxygen concentrations decreased at a constant rate with about 30% of the initial oxygen consumed during each trial. Oxygen consumption rates were estimated using linear regression analyses and expressed as milligrams of oxygen per hour per kilogram of fish (i.e., mg O_2 $kg^{-1}hr^{-1}$). Oxygen consumption rates from six fish were determined for each salinity treatment.

Analytical Procedures

Plasma cortisol levels were determined using a commercial radioimmunoassay kit ([^{125}I] cortisol; GammaCoat, Incstar Corporation, Stillwater, Minnesota) according to Iwama *et al.* (27). Plasma GH, $tPRL_{177}$, and $tPRL_{188}$ levels were measured using the homologous radioimmunoassay developed by Ayson *et al.* (6). Plasma glucose levels were measured using a modification of Trinder's (46) glucose oxidase method (Sigma Chemical Co., St Louis, Missouri). Plasma [Na^+] and potassium ([K^+]) concentrations were measured using an ion chromatograph (Shimadzu Model HIC-6A, Shimadzu Corporation, Kyoto, Japan). Briefly, plasma samples were deproteinated with acetonitrile, diluted with distilled deionized water and injected into the ion chromatograph with 5 mM nitric acid as the mobile phase. Plasma [Cl^-] were determined by coulometric titration (Haake Buchler Instruments digital chloridometer).

Na^+,K^+-ATPase activity (μmoles of ADP mg of protein^{-1}hr^{-1}) in crude gill homogenates was determined at 25°C in a temperature-controlled plate reader (Thermomax, Molecular Devices Corp., Menlo Park, California) according to McCormick (32). In this kinetic assay, the ouabain-sensitive hydrolysis of ATP is enzymatically coupled to the oxidation of NADH, which is directly measured in 96-well microplates at 340 nm for 10 min. Protein content in the gill homogenate was determined using the bicinchoninic acid procedure (43).

Statistical Analysis

Data are presented as means ±1 standard error (SE). Two-way analysis of variance (ANOVA) was used to test for treatment and time effects, whereas oxygen consumption results were analyzed using a one-way ANOVA. Significant treatment means were identified using Student-Newman-Keuls multiple comparison test ($P < 0.05$).

RESULTS

Plasma Cortisol and Glucose Levels

The transfer of fish from the FW stock tank into the 60-l treatment tanks resulted in a significant increase in plasma cortisol titres after 1 day in the FW and ISO groups (Fig. 1A). In contrast, plasma cortisol levels in the SW fish were not elevated 1 day after transfer and were significantly lower than the FW and ISO treatments. Cortisol levels in all three treatment groups were not significantly different at the 4-day sampling period. Plasma glucose levels were significantly higher in FW and ISO on both days 1 and 4, compared to SW values which did not change during the experiment (Fig. 1B).

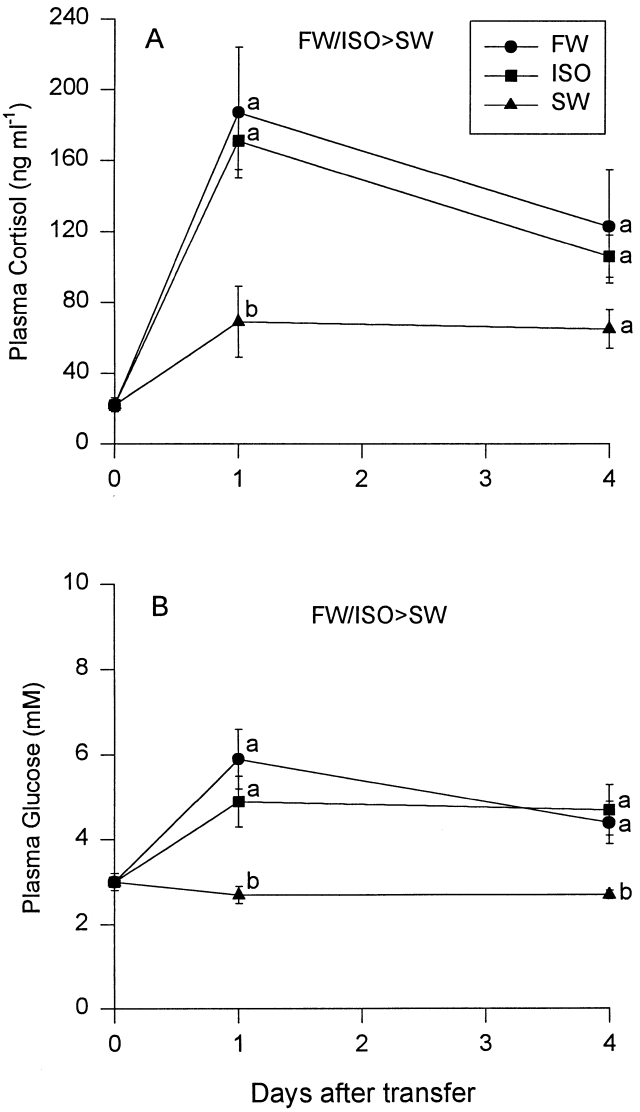

FIG. 1. Plasma cortisol and glucose levels in tilapia (*Oreochromis mossambicus*) after transfer from fresh water (FW) to FW, isosmotic water (ISO) and 75% seawater (SW). Data are presented as means ± SE (n = 6–8). Inset shows significant treatment effects; significant interaction at each time period is shown by letters next to symbols, and means with different letters are significantly different ($P < 0.05$, two-way ANOVA).

Plasma Growth Hormone and Prolactin Levels

Plasma GH levels increased significantly 4 days after transfer to SW, whereas no significant change was observed in

the FW and ISO groups (Fig. 2A). Plasma $tPRL_{177}$ decreased to low levels in SW after 1 and 4 days compared to FW (Fig. 2B). Levels of $tPRL_{177}$ in ISO were intermediate between the FW and SW values. Plasma $tPRL_{188}$ levels were significantly lower in both ISO and SW after 4 days compared to FW (Fig. 2C). The ratio of $tPRL_{188}$:$tPRL_{177}$ was significantly higher in SW (7.0) than in FW (0.9) and ISO (1.0) after 4 days.

Plasma Ion Concentrations and Gill Na^+,K^+-ATPase Activity

Plasma [Na^+] and [Cl^-] in SW were significantly elevated over FW values 1 day after transfer, but returned to FW levels on day 4 (Fig. 3A and C). There was a slight rise in plasma [Na^+] and [Cl^-] after 1 day in ISO. Plasma [K^+] did not differ significantly among salinity treatments at each sampling period (Fig. 3B). Gill Na^+,K^+-ATPase activity of tilapia was significantly greater in SW than in both FW and ISO 4 days after transfer (Fig. 4).

Oxygen Consumption Rates

The average oxygen consumption rate of tilapia 4 days after transfer was significantly (20%) higher in SW than in FW or ISO (Fig. 5). There was no difference in the oxygen consumption rate of tilapia between FW and ISO.

DISCUSSION

The oxygen consumption rate of *O. mossambicus* in FW measured in the present study is comparable with values obtained for this, and other, species of tilapia at a similar temperature, body size, and activity level (1,12,13,19, 28,53). In our study, the metabolic response of *O. mossambicus* to salinity change was measured during the acclimation process, whereas most previous studies have focused on tilapia which have undergone long-term salinity exposure, when all acclimatory processes could be considered complete [e.g., (19,20,28)]. Oxygen consumption rates of *O. mossambicus* in SW (25‰) were significantly elevated 4 days after transfer compared to FW and ISO (12‰). This increase in oxygen consumption rate was associated with an increase in plasma GH levels and gill Na^+,K^+-ATPase activity in SW fish. The oxygen consumption data suggested that the metabolic cost of acclimating to SW after 4 days was at least 20%. This does not necessarily imply that the direct energetic cost of active ion transport processes in osmoregulatory organs such as the gills, intestine, and kidneys was increased by 20%, only that the metabolism of the whole animal was raised by this amount in SW. Increased GH production following SW transfer is likely to stimulate other aspects of metabolism (e.g., increased rates of protein synthesis), in addition to inducing the required osmoregulatory adjustments (e.g., increased gill Na^+,K^+-ATPase activ-

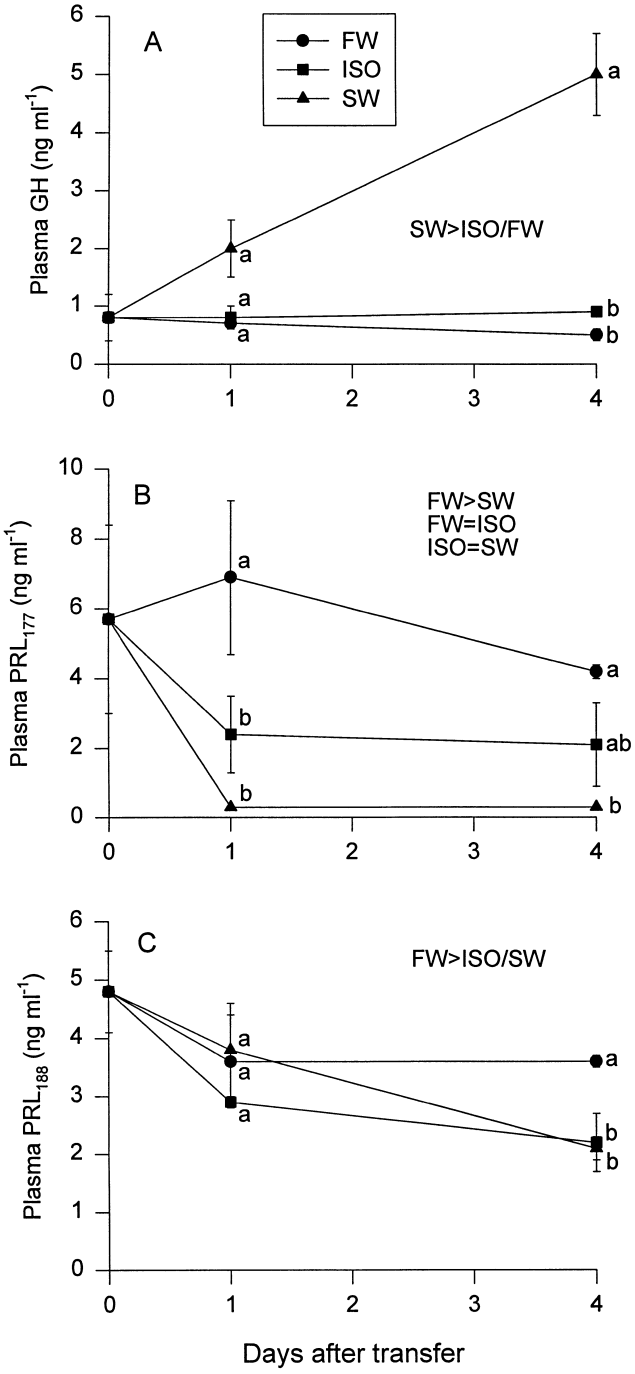

FIG. 2. Plasma growth hormone (GH) and prolactin (PRL_{177} and PRL_{188}) levels in tilapia (*Oreochromis mossambicus*) after transfer from fresh water (FW) to FW, isosmotic water (ISO) and 75% seawater (SW). Data are presented as means ± SE (n = 3–8). Inset shows significant treatment effects; significant interaction at each time period is shown by letters next to symbols, and means with different letters are significantly different ($P < 0.05$, two-way ANOVA).

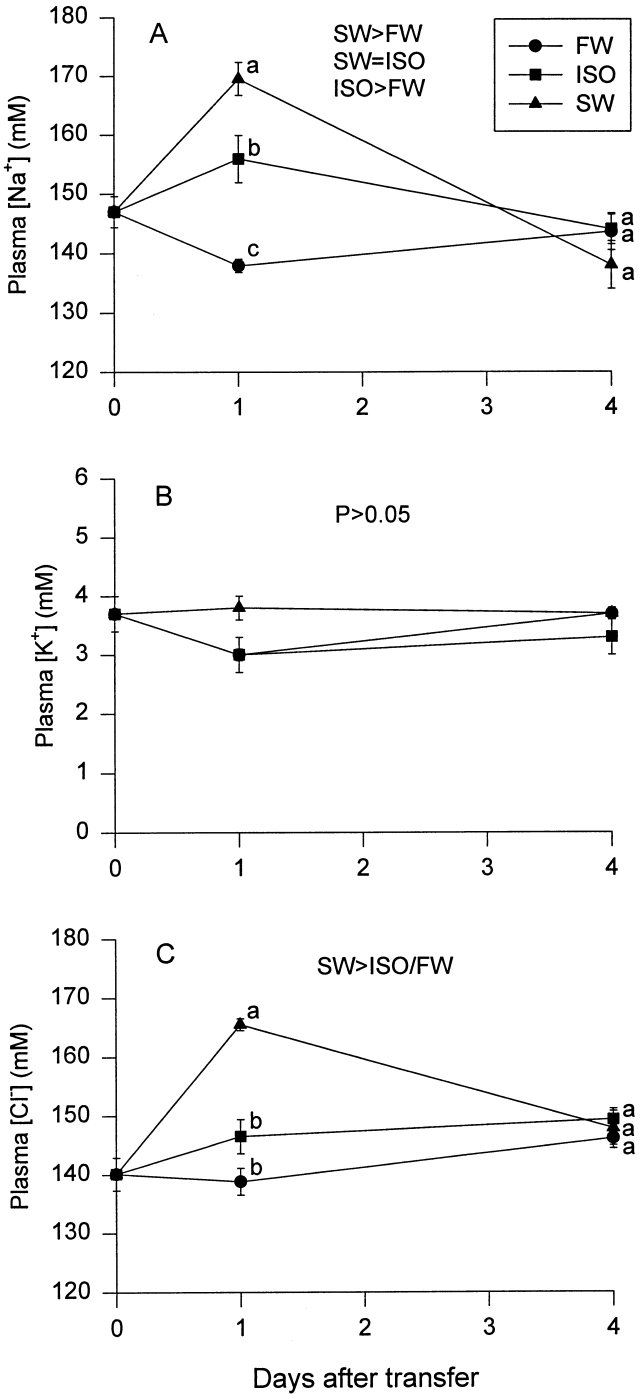

FIG. 3. Plasma [Na^+], [K^+] and [Cl^-] of tilapia (*Oreochromis mossambicus*) after transfer from fresh water (FW) to FW, isosmotic water (ISO) and 75% seawater (SW). Data are presented as means ± SE (n = 6–8). Inset shows significant treatment effects; significant interaction at each time period is shown by letters next to symbols, and means with different letters are significantly different ($P < 0.05$, two-way ANOVA).

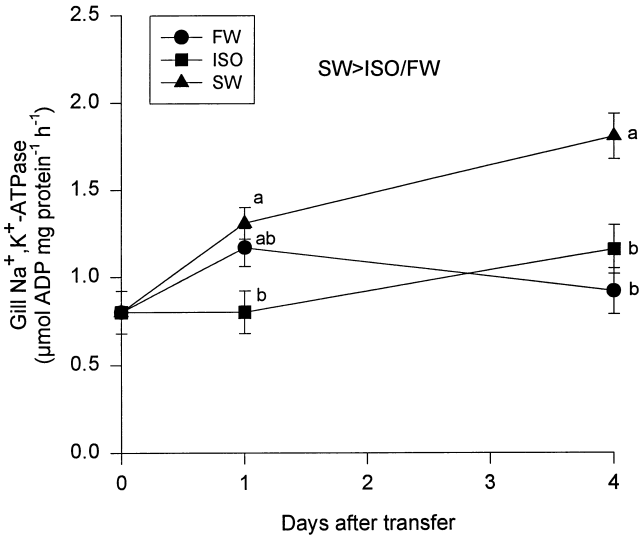

FIG. 4. Gill Na^+,K^+-ATPase activity of tilapia (*Oreochromis mossambicus*) after transfer from fresh water (FW) to FW, isosmotic water (ISO) and 75% seawater (SW). Data are presented as means ± SE (n = 6–8). Inset shows significant treatment effects; significant interaction at each time period is shown by letters next to symbols, and means with different letters are significantly different ($P < 0.05$, two-way ANOVA).

ity). Seddiki *et al.* (42), for example, have recently reported that treatment with trout recombinant GH increased standard oxygen consumption in rainbow trout (*Oncorhynchus mykiss*) by 18% in FW and a further 12% after 4 days in SW. Physiological changes caused by GH, and possibly other hormones, during the SW acclimation process in *O. mossambicus* likely caused the elevated rate of oxygen up-

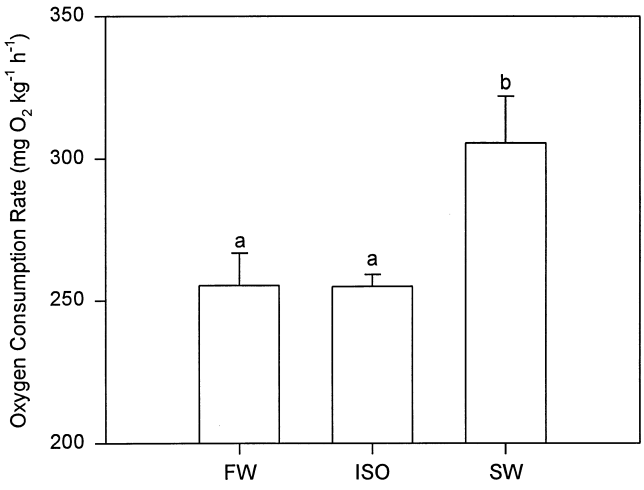

FIG. 5. Oxygen consumption rates of tilapia (*Oreochromis mossambicus*) in fresh water (FW), isosmotic water (ISO) and 75% seawater (SW) 4 days after transfer from FW. Data are presented as means ± SE (n = 6). Means with different letters are significantly different ($P < 0.05$, one-way ANOVA).

take observed in this study. In a related study, we have found that *O. mossambicus* fasted for 1 month could not osmoregulate properly compared to fed fish following a gradual transfer to SW, which also suggests that there is a significant energy requirement for SW acclimation in *O. mossambicus* (48). Further studies are required to delineate the time course of the metabolic response of *O. mossambicus* to SW, by making repeated oxygen consumption measurements throughout the acclimation period.

No difference in the oxygen consumption rate of *O. mossambicus* was observed between FW and ISO in this acute study, suggesting that acclimation to ISO does not impose (or reduce) an energetic demand on this species of tilapia. Febry and Lutz (20) reported that, after a 1-month acclimation, the cost of osmoregulation in Florida red hybrid tilapia, based on differences in oxygen consumption rates, was more expensive in FW than in SW, and was cheapest in ISO. Kültz *et al.* (29) also found that gill Na^+,K^+-ATPase activity was lowest in ISO compared to FW and SW in long-term (5 weeks) acclimated *O. mossambicus*. These results are consistent with the theory that the energetic cost of osmoregulation is lowest in an isosmotic environment, where the ionic gradients between blood and water would be minimal. A recent study by Ron *et al.* (38) found that when *O. mossambicus* were reared from yolksac fry for 20 months in FW or SW, the oxygen consumption rate in SW tilapia was half of that measured in FW fish. Taken together with our data, this suggests that the metabolic cost of acclimating to SW may be high in the short-term, but that in a fully-adapted tilapia the energy requirements of a marine existence may be lower than in FW. It is also now clear that meaningful measurements of oxygen consumption rate in relation to salinity change require a careful consideration of the physiological history of the fish. For example, tilapia which have been reared entirely in SW cannot tolerate direct transfers into FW, whereas FW fish that have been acclimated to SW have no problem making the transition back to FW (E. G. Grau, unpublished observations). It is likely then, that the metabolic cost of SW adaptation in *O. mossambicus* depends to a considerable extent on its individual developmental experience with different osmotic challenges.

Plasma GH levels in *O. mossambicus* increased after transfer from FW to SW, but ISO was not sufficient to trigger an increase in plasma GH. Similar increases in plasma GH levels during SW acclimation have been reported for *O. mossambicus* by Yada *et al.* (49) and for salmonids by a number of studies [reviewed by (40)]. Borski *et al.* (11) found that tilapia reared in SW for 7 months from the yolksac stage had more active GH cells in their pituitaries than did FW fish, even though plasma levels may not be elevated long-term [3–4 weeks; (6)]. Work with salmonids suggests that the initial rise in plasma GH levels is followed by a concomitant increase in the metabolic clearance rate of GH (39,41). The chronic elevation of GH cell activity in SW tilapia may help to explain the higher growth rates observed in SW tilapia compared to FW fish (30,38). Plasma levels of the two PRLs in the present study declined after transfer from FW to SW; this is in agreement with previous observations in *O. mossambicus* transferred from FW to SW (6,35,49). The reduction of plasma PRL levels after SW transfer is related to its sodium-retaining action (15), which would be counterproductive to maintaining proper ionic balance in SW fish. The concentration of $tPRL_{177}$ was somewhat intermediate in the ISO treatment compared to FW and SW, and a similar progressive response to salinity was seen in total PRL levels by Nicoll *et al.* (35). The higher plasma $tPRL_{188}$:PRL_{177} ratio observed in SW in this study suggests that the two prolactins may be differentially regulated during SW acclimation, as proposed earlier by Yoshikawa-Ebesu *et al.* (51) using *in vitro* pituitary preparations of *O. mossambicus*.

Gill Na^+,K^+-ATPase activity of *O. mossambicus* in SW increased 2.2-fold over the FW value after 4 days. A similar result was reported by Hwang *et al.* (26), who found that it took gill Na^+,K^+-ATPase activity of *O. mossambicus* 2 days to increase significantly (1.3-fold) above the FW control after transfer to 20‰ salinity. The timing of the decrease in plasma [Na^+] and [Cl^-] and the increase in gill Na^+,K^+-ATPase activity in the present study are consistent with the known role of this enzyme in salt secretion to maintain ionic balance in SW fish (52). GH treatment has recently been found to increase gill Na^+,K^+-ATPase activity in *O. mossambicus* (11) and the increase in both plasma GH levels and enzyme activity during SW acclimation in the present study lends further support to a possible osmoregulatory role for GH in *O. mossambicus*. In contrast, GH does not appear to increase the adaptability of a related species, the Nile tilapia (*O. niloticus*), to brackish water (5). Transfer to ISO did not have a significant effect on gill Na^+,K^+-ATPase activity after 4 days in this study. Dange (16) showed that a salinity of at least 17‰ was required to increase gill Na^+,K^+-ATPase activity of *O. mossambicus* 1 week after transfer.

Plasma cortisol levels in FW and ISO were significantly elevated 1 day after transfer. This was probably a result of the stress associated with being transferred into the smaller treatment tanks. Assem and Hanke (4) found that plasma cortisol levels in *O. mossambicus* transferred to 27‰ salinity increased significantly above the FW value at 2 hr and returned to normal 6 to 72 hr after transfer. In contrast, we found that plasma cortisol levels in the SW fish were significantly lower than the FW and ISO treatments 24 hr after transfer. The lower plasma cortisol levels in SW fish after 1 day may have been related to an increase in the clearance rate of cortisol, rather than a decrease in cortisol secretion. Nichols and Weisbart (34), for example, reported that plasma cortisol concentrations in Atlantic salmon (*Salmo salar*) were significantly lower after transfer to SW, and that the metabolic clearance rate of cortisol was significantly higher in SW compared to FW. Balm *et al.* (8) have recently shown that cortisol production in SW tilapia was several-fold higher than in FW fish, even though plasma

cortisol levels between the two groups were similar, again suggesting an increase in clearance rate. Redding *et al.* (37) have further demonstrated that gills of SW-adapted coho salmon (*Oncorhynchus kisutch*) take up and retain more cortisol than do gills of FW fish. Therefore, during the initial stages of the SW acclimation process in *O. mossambicus*, it is possible that an increased release of cortisol in the blood is soon followed by an enhanced uptake by osmoregulatory organs. Plasma glucose levels followed the same pattern as cortisol, being elevated in FW and ISO compared to SW. Assem and Hanke (3) also found glucose concentrations in *O. mossambicus* to increase in FW for 6 hr due to transfer stress, but levels in SW were elevated above FW values for up to 24 hr after transfer. As in cortisol, the lower glucose levels in SW in the present study may have reflected an elevated uptake by metabolizing cells, but a glucose turnover study would be necessary to verify this. Plasma protein levels and hematocrit values did not change with increasing salinity (data not shown), thus the lower plasma glucose and cortisol concentrations in SW observed in this study were unlikely to be due to plasma volume changes.

Following transfer from FW to SW, plasma [Na^+] and [Cl^-] peaked after the first day and declined to FW levels (about 145 mM) on day 4. Similar patterns for these ions were observed by Assem and Hanke (2) and Hwang *et al.* (26) after direct transfer of *O. mossambicus* from FW to 27 and 20‰ salinity, respectively. Plasma [Na^+] and [Cl^-] were slightly elevated in 12‰ after 1 day, and although this salinity is generally considered to be isosmotic for tilapia (19,20), [Na^+] and [Cl^-] in ISO were, in fact, higher than in the plasma of FW fish (Table 1). It is the presence of organic osmolytes, such as glucose and albumin, in the plasma that gives it the same osmotic pressure as 12‰ salinity water. Plasma [K^+] did not change significantly after transfer to SW, which was also observed for *O. mossambicus* by Assem and Hanke (2). This is possibly due to the lower gradient for K^+ between the blood and SW compared to Na^+ and Cl^-. Alterations in plasma [K^+] can also be buffered by transfer to and from the large intracellular pool of K^+ and therefore may not pose a problem for regulation compared to Na^+ and Cl^- (33).

In conclusion, the SW acclimation process in *O. mossambicus* involves several hormonal and osmoregulatory adjustments in order to re-establish ionic homeostasis. The present study demonstrates that these physiological changes represent a significant short-term energetic cost, elevating metabolic rate by about 20% after 4 days in SW. In contrast, acclimation to ISO did not require substantial physiological adjustment, and after acute exposure did not impose (or reduce) an energetic demand in *O. mossambicus*.

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